STATEMENT OF WORK

for

"Development of Molecularly Imprinted Polymers for the Detection of Pyrethroids"

Background:

The U.S. Environmental Protection Agency (EPA), National Exposure Research Laboratory/ Human Exposure & Atmospheric Sciences Division (HEASD), Exposure & Dose Research Branch (EDRB), Las Vegas, Nevada, is interested in developing molecularly imprinted polymers (MIPs) to detect pyrethroid pesticides. MIPs may be cost-effective reagents for analytical methods such as affinity chromatography sample preparations, biosensors, and competitive assays. The suitability of the MIPs as antibody replacements in analytical methods must be determined.

Molecular imprinting is a technique to develop polymers that can mimic the function of antibodies. Monomers are initially formed that can interact with the analyte through mechanisms such as hydrogen bonding. These monomers are then polymerized in the presence of the non-covalently interacting analytes. After polymerization, the analyte molecules are removed through washing leaving 3D cavities which resemble the shape of the analyte molecules. The final polymeric product recognizes the analyte based on the interactive forces and the created imprinted shape.

Rapid and cost-effective analytical methods are needed for environmental monitoring and human exposure assessment studies. One way to reduce uncertainty in the assessment of human exposures to environmental contaminants is to better characterize potential routes of exposures. Such studies depend on the analysis of multiple samples and often multiple analytes. Effective on-site environmental monitoring often requires methods that are rapid, inexpensive and easy to perform. Faster and more cost-effective field screening and monitoring methods can increase the amount of information available concerning the presence and concentration of contaminants that might impact human health and the environment.

Immunoaffinity chromatography and solid phase extraction (SPE) techniques can provide efficient sample preparations for instrumental analyses such as high-performance liquid chromatography (HPLC). Molecularly imprinted polymers (MIPs) may provide advantages when used as stationary phases for affinity chromatography or SPE. MIPs have recently been used for the determination of single analytes such as atrazine and tamoxifen and for the multi-analysis of triazine and phenylurea compounds. The application of MIPs in detection methods based on enzyme-linked immunosorbent assays appears feasible but has yet to be fully investigated.

MIPs for related pyrethroid pesticides will be developed and their suitability as antibody replacements in competitive assays for environmental monitoring and human exposure assessment methods will be determined.

Objective Statement:

Contractor shall develop MIPs with selective binding to pyrethroid pesticides. A class-selective approach as well as a single-analyte approach shall be followed for the determination of similar pyrethroids including: allethrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, fluvalinate, permethrin and tetramethrin**.** Structural information on the pyrethroids of interest shall be obtained prior to MIP development. Contractor shall evaluate MIPs for the detection of the pyrethroids. Contractor shall develop standard operating procedures for each method developed. Contractor shall develop a Category 4 Quality Assurance Project Plan (QAPP).

Task Descriptions:

I. Category 4 Quality Assurance Project Plan (QAPP):

Contractor shall develop and deliver an acceptable Category 4 QAPP. See attached "Review Criteria for Category 4 QAPP" for guidance. The plan shall be delivered to the EPA Project Officer before initiation of laboratory work.

II. Surface Imprinting:

Contractor shall employ surface imprinting technology such as electrochemical transduction (or optical detection) methods to obtain data on the structural features of the pyrethroids of interest prior to MIP development. The eight pyrethroids of interest are: allethrin, permethrin, cypermethrin, deltamethrin, tetramethrin, fenvalerate, cyfluthrin, and fluvalinate. It is anticipated that some of the MIPs developed would be able to detect more than one of the pyrethroid compounds. In addition, a single-analyte approach is to be investigated for permethrin. Contractor shall apply functional surfactants to differentiate the recognition capabilities of the cavities. Contractor shall use modified ITO electrodes for detection of the acid metabolites, i.e., hydrolysis products of the pyrethroid ester bond including *cis-trans*-DBVA.

The parameters to be examined are:

- cavity formation by pyrethroids in self-assembled monolayers
- concentration effect
- evaluation of the hydrolysis products
- quality of the monolayer components to enhance specificity

III. Molecularly Imprinted Polymers (MIPs):

Contractor shall investigate the potentiality of applied structural features involved with amphiphilic properties of both components. Contractor shall first investigate these properties on surfaces in Task II above and then transfer this knowledge to amphiphilic polymers such as alkylated polymers for MIP preparation. Contractor shall investigate the ability of the analyte molecules to create cavities in the formed layer-like structures. Contractor shall investigate how to chemically modify the structural components to introduce more specific interactions as required.

Contractor shall apply a layer-by-layer technology to capture the recognized molecules within the layers on a molecular level based on the pyrethroids potential for ionic interactions.

The parameters to be examined are:

- amphiphilic cavity-structure forming polymers
- ionic structure forming polymers
- MIPs with specific interactions (H-bonding, bivalent ions)

IV. Transduction detection methods:

Potentiometric detection will be applied to the imprinted surfaces to evaluate method sensitivity. Ion channel amplification should be used when possible to increase sensitivity.

Fluorescence-based methods shall be developed with two alternative approaches based on:

- the optically active molecular component of the initial interacting monomer
- the optically active component in the backbone of the polymer chain.

Methods shall be optimized for sensitivity.

Milestones:

First quarter. Development of a QAPP. The interactive properties of the analytes shall be investigated. Apply structure formation to assist the imprinting efficiency.

Second quarter. Select polymeric system and prepare MIPs.

Third quarter. Determine selectivity of prepared MIPs for analyte detection. Draft of SOPs.

Fourth quarter. Optimize detection methods to maximize sensitivity.

Deliverables and Schedule:

Task 1: Delivery of a Category 4 QAPP before initiation of laboratory work - within 30 days after award of order.

Task 2: Delivery of at least 3 molecularly imprinted polymers (500 mg each) for the detection of pyrethroids - 12 months after award of order.

Task 3: Delivery of standard operating procedures for MIP development - draft due within 9 months and final due 12 months after award of order. This task will be considered 80% complete upon acceptance of draft; 100% at acceptance of the final report of the standard operating procedures for MIP development.

Task 4: Delivery of standard operating procedures for analyte detection - draft due within 9 months and final due 12 months after award of order. This task will be considered 75% complete upon acceptance of draft; 100% at acceptance of the final report of the standard operating procedures for analyte detection.

Task 5: Delivery of Final Report - 12 months after award of order.

Acceptance Criteria:

- Quality Assurance documentation for the development of each MIP
- Standard Operating Procedures (SOPs) for each MIP developed
- Standard Operating Procedure (SOP) for each analytical method developed

Special Reporting:

-- Quality Assurance documentation (including QAPP)
- Standard Operating Procedures (SOPs) for MIP development and analyte detection
- Final Report describing each phase of the project, e.g., development, optimization, and evaluation of MIPs and related analytical methods